UNIVERSITÉ DU QUÉBEC À MONTRÉAL

EFFETS COMBINÉS DE LA CONSANGUINITÉ ET DE L'ENRICHISSEMENT SUR LA STÉRÉOTYPIE DE SOURIS SYLVESTRES (*PEROMYSCUS MANICULATUS SONORIENSIS*)

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AVANT-PROPOS

Nous avons choisi de présenter ce mémoire de maîtrise sous la forme d'un article scientifique. Celui-ci sera soumis sous le titre de « Inbreeding and enrichment: effects on stereotypy in captive deer mice (*peromyscus maniculatus sonoriensis*)». Les auteurs de l'article seront Leïla Turki (UQAM), France Landry et Denis Réale (UQAM). L'élevage des souris et la mise en place du protocole expérimental ont été assurés par Leïla Turki et France Landry sous la supervision de Denis Réale. La récolte des données, l'analyse statistique des résultats, la rédaction ainsi que la recherche bibliographique qui ont servi à cette étude ont été effectuées par Leïla Turki sous la supervision de Denis Réale.

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A	Age group
BLUP	Best Linear Unbiased Predictors
C	Cage type
e.g.	Par exemple (du latin *exempli gratia*)
df	Degrees of Freedom
E	Enrichment environment
et al.	Et autres (du latin *et alii*)
F	Inbreeding coefficient
Fig.	Figure
G	Generation
h^2	Heritability
ID	Identity
I	Inbreeding
i.e.	C'est-à-dire (du latin *id est*)
Loglik	Log-likelihood
LR	Log-likelihood Ratio
LRT	Log-likelihood Ratio Test
NB	Number of individuals
O	Outbred
REML	Restricted Maximum Likelihood
Se	Sex
S	Standard environment
V_A	Additive variance
V_P	Phenotypic variance

RÉSUMÉ

Deux problèmes importants caractérisent les conditions d'élevage en captivité (animaux domestiques, de laboratoire, de zoo): la reproduction consanguine, qui rend les animaux plus fragiles et plus sensibles au stress, et la pauvreté des stimuli environnementaux, qui accroît le stress et affecte la santé et le bien-être des animaux. Un indice de faible bien-être ou de stress chez un animal maintenu en captivité est l'apparition et le maintien de comportements stéréotypiques. Ceux-ci se caractérisent par des mouvements répétitifs, sans buts apparents. Les individus d'une même espèce ne réagissent pas nécessairement de la même façon à des mêmes conditions, et les animaux très actifs semblent avoir une plus grande propension à développer des comportements stéréotypiques en captivité. Nous testons sur deux générations de souris sylvestres (*Peromyscus maniculatus sonoriensis*) sauvages maintenues en laboratoire l'effet combiné de la consanguinité et de l'enrichissement (social et/ou physique) sur l'activité, et de l'effet combiné de la consanguinité, de l'enrichissement (social et/ou physique) et de l'activité sur l'expression de la stéréotypie. Celle-ci apparaît chez la souris entre 20 et 90 jours suivant la naissance et continue de se manifester à l'âge adulte. Nous estimons par la suite la répétabilité de l'activité et de la stéréotypie. Quatre groupes de souris ont été formés à chaque génération : des consanguins (F entre 0,25 et 0,34) en milieu riche (enrichissement physique) ; des consanguins en milieu pauvre (conditions d'habitation standard) ; des non-consanguins (F entre 0,05 et 0,078) en milieu riche ; des non-consanguins en milieu pauvre. La présence de différents comportements stéréotypiques a été notée au cours de 28 périodes d'observation, de cinq minutes chacune, par individu, pendant l'ontogenèse de la stéréotypie. Par la suite, chaque souris a été observée pendant 7 à 9 jours non consécutifs toutes les demi-heures pendant 7 heures. Des tests linéaires mixtes généralisés indiquent que la consanguinité peut interagir avec l'enrichissement de sorte à affecter l'activité générale dans la cage ou les comportements stéréotypiques, mais que les effets ne sont pas nécessairement tranchés ni permanents : ils peuvent varier entre les générations et, à long terme, au sein d'une même génération. Par ailleurs, l'enrichissement social ou physique réduit plus la propension des souris très actives à devenir stéréotypiques que celle des souris peu actives. Enfin, les modèles linéaires mixtes et les BLUPs (best linear unbiased predictors) calculés à partir de ces derniers indiquent que l'activité et la stéréotypie sont très répétables : elles sont très influencées par l'appartenance à la famille chez les juvéniles ; avec l'âge, ce sont les effets individuels qui deviennent le plus marqués.

Mots-clés : souris sylvestres, stéréotypie, tempérament, activité, consanguinité, enrichissement social ou physique.

INTRODUCTION GÉNÉRALE

La consanguinité est un phénomène répandu tant chez les animaux sauvages que chez les animaux maintenus en captivité et suscite, notamment, des questions relatives à la conservation des espèces et à leur bien-être. Un nombre croissant d'études montre que ses effets peuvent être exacerbés par un environnement stressant, et affecter des traits reliés à la fitness. On sait toutefois peu de chose sur son impact sur les comportements, particulièrement dans un contexte de stress environnemental. Or, la consanguinité et la pauvreté des stimulations physiques ou sociales, source de stress, sont deux facettes importantes de la gestion des espèces maintenues en captivité. Des carences affectives et des conditions de dénuement peuvent, en effet, limiter l'expression de comportements normaux et favoriser le développement de comportements répétitifs anormaux, dits stéréotypiques. Il a été montré, toutefois, qu'il existe au sein d'une même espèce des variations interindividuelles liées à la propension à devenir stéréotypique, et ces variations pourraient en partie être expliquées par des différences de tempérament.

1.1 La consanguinité

Les individus consanguins sont plus susceptibles d'être homozygotes pour des allèles récessifs délétères ou létaux. Ils courent davantage le risque de souffrir de dépression consanguine, définie comme une altération (généralement une réduction) de la valeur phénotypique moyenne d'un caractère due à la consanguinité (Falconer 1996). Cette altération est généralement censée être associée à une diminution de leur valeur adaptative (*fitness*). La dépression consanguine touche des composantes biodémographiques des individus concernés, notamment leur fertilité, leur viabilité, ainsi que leur résistance à la maladie (Charlesworth et Charlesworth 1987 ; Roff 1998). Sur le plan physique, elle affecte souvent le poids à la naissance et la croissance ; elle est la source d'anomalies du développement ou de malformations physiques (Margulis et Altmann 1997 ; Margulis 1998).

La plupart des études évaluent certains effets à court terme de la consanguinité ou apparaissant autour de la naissance. Cependant, la survie néonatale ou à des stades précoces

de la vie n'est pas le seul moyen de mesurer l'effet de la consanguinité. Les individus consanguins qui parviennent à l'âge adulte peuvent manifester de la dépression consanguine sur le plan de certaines composantes de la valeur adaptative exprimées à des stades plus tardifs de la vie (Sharp 1984 ; Charlesworth et Charlesworth 1987 ; Margulis et Altmann 1997). Par exemple, leurs comportements ou leur physiologie peuvent en être affectés de sorte à inhiber ou à rendre leur reproduction difficile. Ils risquent aussi d'avoir une progéniture déficiente (même si celle-ci n'est pas consanguine) ou de ne pas réussir à l'élever (Margulis et Altmann 1997 ; Margulis 1998). Il a été démontré récemment, par exemple, que la dépression consanguine pouvait avoir un impact sur le plan comportemental. Ainsi, elle peut affecter les interactions entre les parents et leur progéniture, notamment les comportements maternels (Margulis 1997), les niveaux d'activité après la mise en couple (Margulis et Altmann 1997 ; Margulis 1998 ; Meffert, Hicks et Regan 2002), la parade nuptiale et la motivation sexuelle mâle (Aspi 2000 ; Mariette *et al.* 2006), et la capacité de compétition pour obtenir un territoire (Höglund *et al.* 2002).

Par ailleurs, il semble que les effets de la consanguinité soient exacerbés dans des conditions environnementales difficiles, comme des températures extrêmes, la présence de toxiques dans le milieu, des niveaux d'acidité ou de sel très élevés, etc. (Frankham 1995; Falconer & Mackay 1996; Roff 1998; Bijlsma *et al.* 1999; Meagher *et al.* 2000; Kristensen *et al.* 2003; Reed *et al.* 2003). La consanguinité réduirait la capacité à répondre à un stress environnemental (voir Miller 1984; Keller et Waller 2001, Keller et al. 2002; Armbruster et Reed 2005; Szulkin et Sheldon 2007), et les hybrides auraient une valeur adaptative supérieure (hétérosis) dans des conditions environnementales stressantes. Plus encore, il semble que l'étendue de l'hétérosis croisse à mesure que les conditions deviennent plus dures (voir Miller 1994).

1.2 Notions de persistance des comportements et personnalité

Aujourd'hui, en biologie, un groupe croissant de chercheurs s'arrête sur le fait que des individus évoluant dans un même milieu peuvent réagir différemment aux mêmes conditions. Il s'intéresse au sens des variations interindividuelles et intra-individuelles au fil du temps et

au sein d'une espèce. La question de la répétabilité a contribué à cet intérêt. Boake (1989) a soulevé la question de la signification de la variation intra-individuelle et, plus particulièrement, de l'importance de comprendre à quel point les traits des individus sont constants-cohérents, soit « répétables », au fil du temps. Comme il le relève, pour évaluer notamment la valeur sélective d'un trait, il faut comprendre à quel point il est variable entre les individus et au sein d'un même individu (Hayes et Jenkins 1997). Les termes personnalité, tempérament, coping style décrivent généralement un phénomène commun : le fait que des différences comportementales individuelles sont, dans une certaine mesure, persistantes (Budaev 1997 ; Box 1999 ; Gosling 2001).

Parler de persistance des traits (ou, plus précisément, de persistance des différences phénotypiques interindividuelles) ne signifie pas que ceux-ci ne se modifient pas au fil du temps. Ils ne sont pas parfaitement répétables pour deux raisons majeures : 1) ils sont affectés par l'âge (ex. : la fécondité peut changer avec l'âge) ; 2) ils sont affectés par l'environnement (ex. : les chameaux peuvent altérer leur température corporelle et les taux d'échange de l'eau et de la chaleur en fonction de la disponibilité de l'eau) (Hayes et Jenkins 1997). La répétabilité implique plutôt que la différence entre les individus tend à se maintenir (Réale *et al.* 2007). Cette cohérence individuelle résulterait d'une combinaison d'effets génétiques, épigénétiques et environnementaux (Sih *et al.* 2004b). Une bonne évaluation de la variation individuelle nécessite donc qu'on comprenne à la fois comment certaines caractéristiques varient selon les individus et comment elles changent dans le temps chez un même individu (Hayes et Jenkins 1997).

1.2.1 Personnalité humaine, traits de tempéraments animaux

1.2.1.1 Personnalité ou tempérament chez les humains

La plus grande partie des recherches théoriques et empiriques portant sur la personnalité a trait à l'humain et a été menée par des psychologues ou encore par des généticiens du comportement. Ces derniers se fondent beaucoup sur des modèles de génétique quantitative, qui supposent que la variance de traits quantitatifs est influencée par un grand nombre de

gènes, chacun exerçant de petits effets individuels. Cela est appuyé par des études récentes montrant que les **QTL** (*quantitative traits loci*) contribuent généralement à moins de 10 % de la variance totale d'un trait. Les traits de tempérament appartiendraient à cette catégorie (Flint 2002 ; Flint 2003).

Cependant, les psychologues de la personnalité humaine étudient différents phénomènes, comme le tempérament, les traits de caractère, les dispositions, les buts, les humeurs, les histoires de vie... Aucune définition particulière de la personnalité n'est capable de répondre aux critères de tous les psychologues de la personnalité. Pour ce qui est du tempérament, en psychologie, c'est un concept proche de la personnalité : les théoriciens s'accordent souvent sur le fait que c'est une composante de la personnalité et qu'elle a un sens plus restreint. Cependant, ils ne s'entendent pas sur la délimitation de ces deux concepts (Feist 1994). Le tempérament peut renvoyer à des différences d'émotivité ou à des traits apparus très tôt dans l'ontogenèse (Budaev 1997 ; Box 1999), ou aux tendances héritées, apparaissant tôt dans la vie, qui se maintiennent tout au long de la vie et servent de fondement de la personnalité (Goldsmith *et al.* 1987). Par ailleurs, de grandes différences sont au cœur même de la psychologie de la personnalité. Ainsi, certains théoriciens traitent l'héritabilité comme un critère qui rend les différences individuelles majeures (Eysenck 1981, cité dans Buss et Greiling 1999). D'autres ne tiennent pas compte de la question de l'origine héritable ou non (Saucier et Goldberg 1996), ou supposent simplement que les différences les plus importantes naissent de l'expérience pendant l'ontogenèse (McClelland 1980).

1.2.1.2 Chez les animaux...

Les chercheurs travaillant sur les animaux ne s'entendent pas plus sur une définition des concepts de personnalité et de tempérament. Les premières tentatives pour évaluer la personnalité animale (comme les études pionnières de Robert Yerkes et de Stevenson-Hinde) ont été menées dans les années 70-80. Des patrons de variations individuelles comportementales analogues à ceux des humains ont été observés chez des primates, des animaux domestiques, des rongeurs de laboratoire, des oiseaux, des reptiles, des poissons, des mollusques et des arthropodes (voir la revue de littérature de Gosling et John 1999 ; Gosling 2001). Ces différences comportementales ne peuvent être uniquement expliquées par

le sexe, l'âge ou d'autres caractéristiques individuelles, comme le statut reproducteur ou le niveau de satiété (Sih *et al.* 2004b). Chez les humains, ces différences ont été appelées « types de personnalité ».

Bien qu'il n'y ait rien, dans la théorie de l'évolution, qui suggère que seuls les traits morphologiques sont soumis aux pressions de sélection, et que la continuité évolutive entre les humains et les autres animaux indique que certaines dimensions de la personnalité sont communes à une grande gamme d'espèces, les biologistes hésitent à attribuer des traits de personnalité, des émotions et des *cognitions* aux animaux. Même s'ils conçoivent que l'anatomie et la physiologie des humains soient similaires à celles des animaux (Gosling 2001), ils hésitent à apposer à ceux-ci des étiquettes psychologiques et à appliquer certaines approches communes dans les études portant sur les humains, mais difficiles, voire impossibles, à intégrer dans les recherches sur des espèces où la communication par langue et l'introspection sont irréalisables (Groothuis et Carere 2005). On a appelé les patrons de variations individuelles animales « personnalité », « tempérament », « tendances comportementales » ou « stratégies comportementales », « syndromes comportementaux », « profils comportementaux », « *coping styles* » ou « *coping strategies* »... Les deux premiers termes ont une connotation liée à la classification des personnalités humaines. Parfois, le terme « tempérament » semble utilisé juste pour éviter l'emploi de « personnalité », que certains associeraient à de l'anthropomorphisme (Gosling 2001). L'existence de différents tempéraments peut jouer un rôle fonctionnel : des phénotypes distincts au sein d'une espèce pourraient avoir une fitness différentielle selon des conditions environnementales variées, comme la densité de population, la stabilité sociale, la disponibilité de nourriture, etc. (Koolhaas *et al.* 1999).

Réale et *al.* (2007) considèrent le tempérament et la personnalité comme des synonymes. Selon ces auteurs, de nombreuses définitions proposées renvoient à des concepts difficiles à observer ou à mesurer, et le tempérament devrait être défini de façon opérationnelle : il s'agit de concepts permettant de regrouper des patrons de comportements ou des dispositions individuelles relativement persistantes qui sous-tendent et modèlent l'expression du comportement. Celles-ci doivent être distinguées des états de motivation (comme le degré de faim) et de traits d'aptitude (comme les capacités cognitives), bien qu'une interaction entre

ces facteurs soit impliquée dans l'expression d'un patron comportemental. Par exemple, un individu dont l'aptitude compétitive est faible peut être poussé par la faim à devenir plus explorateur ou moins néophobe, ou à prendre plus de risques qu'il ne le ferait normalement. Cette cohérence individuelle résulte d'effets génétiques, épigénétiques et environnementaux combinés. Selon cette définition, le tempérament peut être mesuré grâce à des indices physiologiques, hormonaux ou comportementaux.

Réale et *al.* (2007) divisent les traits de tempérament en cinq catégories utiles pour les écologistes en tant qu'outils : 1) la hardiesse, soit la réaction d'un individu à une situation risquée, mais pas nouvelle (ex. : réaction face à des prédateurs, à des humains...) ; 2) l'exploration, qui inclut la néophobie ou la néopholie, et qui est la réaction d'un animal à une situation nouvelle (ex. : un nouvel habitat, une nouvelle nourriture, etc.) ; 3) l'activité, soit le niveau d'activité général d'un individu (ce trait peut interférer avec les mesures d'exploration ou de hardiesse) ; 4) la sociabilité, soit la réaction d'un animal à la présence ou à l'absence de congénères (hormis les comportements agressifs) ; 5) l'agressivité, soit les réactions agonistes envers des congénères. Chaque trait de tempérament occupe comme un continuum. Par exemple, la hardiesse et la timidité sont les deux expressions extrêmes de la hardiesse).

Les traits devraient : 1) montrer une certaine variation phénotypique ; 2) être « répétables » (indiquant la persistance du comportement d'un individu) ; 3) être héritables (l'héritabilité d'un trait est la proportion de variance phénotypique expliquée par la variance génétique additive du trait ; elle représente son potentiel évolutif, soit sa capacité à changer de moyenne et de distribution au fil des générations sous l'effet de la sélection). Les valeurs individuelles des mesures d'un trait sont appelées phénotypes.

1.3 Tempérament, captivité et stéréotypie

Certains traits de tempérament semblent être liés à des comportements répétitifs anormaux observés chez des animaux captifs de différentes espèces — que ce soit des animaux de zoo, d'élevage ou de laboratoire — et désignés traditionnellement par le terme stéréotypie. Celle-ci est communément définie comme « des patrons de comportements répétitifs, généralement

invariables et dépourvus d'une fonction apparente » (Ödberg 1986 ; Mason 1991 ; Sprague et VandenBos 1996 ; Wiedenmayer 1997). Il a été noté que les animaux ont des propensions différentes à développer de la stéréotypie dans des conditions de captivité similaires pour des raisons encore mal comprises. Une des explications avancées est qu'ils diffèrent relativement à des traits comportementaux importants sur le plan écologique, comme l'activité et l'exploration, et que cela se répercute sur la façon dont ils réagissent aux conditions de captivité. Ainsi, la stéréotypie a été associée à des différences dans le niveau général de l'activité ou de l'exploration, dans la taille du domaine vital (*home range*), etc. Par exemple, des souches plus actives de rongeurs élevés en captivité ont davantage tendance à développer de la stéréotypie (Würbel et Stauffacher 1994, cités dans Würbel 2006). Au sein d'une même espèce, les individus les plus actifs ont plus de chances de devenir stéréotypiques (souris et campagnols roussâtres, Ödberg 1986, cités dans Cooper et Nicol 1996 ; Bildsoe *et al.* 1991). Chez les carnivores, les espèces ayant un domaine vital étendu ont une plus grande propension à devenir stéréotypiques en captivité que les autres (Clubb et Mason 2003) ; même chose en ce qui concerne les espèces plus néophiles, comme les ours, ou chez les individus d'une espèce ayant une plus grande motivation à explorer (Poole 1998, cité dans Clubb et Vickery 2006).

1.3.1 Stéréotypie et conditions environnementales

Beaucoup de chercheurs se sont penchés sur les conditions environnementales conduisant à l'apparition de la stéréotypie. Ils considèrent souvent qu'elle naît en réponse à des situations physiques et sociales non favorables (Mason 1991 ; Wiedenmayer 1997). Elle serait l'indicateur d'une forte détresse ou d'un faible bien-être passés ou présents des animaux maintenus en captivité. Elle se manifeste souvent chez ceux qui font face à des problèmes insolubles de trois sortes : premièrement, à des conditions de frustration chronique, dans lesquelles ils sont fortement motivés à effectuer un comportement, mais sont dans l'incapacité de le faire (Broom 1991). Cela les amène souvent à rediriger leurs activités (Rushen et *al.* 1993). Ainsi, un environnement artificiel comme une cage peut limiter ou empêcher la réalisation d'un comportement normal, ce qui élève le niveau de stress de l'animal captif. Il a été montré, par exemple, qu'un animal fouisseur (comme une gerbille de

Mongolie, *Meriones unguiculatus*) qui est motivé à se cacher sans pouvoir le faire est amené à creuser même s'il ne peut construire de tanière (Wiedenmayer 1997). Deuxièmement, la stéréotypie peut se développer lorsqu'un animal fait face à des situations conflictuelles, apeurantes ou stressantes, qui sont récurrentes (Meyer-Holzapfel 1968 ; Kiley-Worthington 1977), comme une alimentation limitée (Schoenecker et Heller 2000) ou des chocs électriques sans issue. Enfin, des conditions restrictives sur le plan du mouvement ou de l'espace, ou non stimulantes sur le plan sensoriel ou exploratoire, peuvent susciter l'apparition de comportements stéréotypiques.

Pour pallier ces différentes causes possibles de stéréotypie, il a été suggéré d'enrichir le milieu captif. La définition typique d'un enrichissement, c'est « la combinaison d'une stimulation inanimée et d'une stimulation sociale » (Rosenzweig, 1978). Généralement, les animaux « enrichis » sont maintenus dans un environnement plus grand et plus complexe que ceux qui sont élevés dans un milieu standard. Ils ont l'occasion de pratiquer volontairement de l'activité physique (par exemple, sur une roulette dans le cas de rongeurs) et d'interagir socialement (dans le cas d'animaux sociaux). Pour les stimuler, on les expose à quelques éléments de variabilité. Un milieu pauvre physiquement et un contexte de carence sociale favorisent la stéréotypie, peut-être parce que la stimulation sensorielle est en elle-même nécessaire (Petrie 1986, cité dans Mason 1991), ou parce que le degré d'excitation doit être maintenu dans certaines limites (Hennessy et Levine 1979, cités dans Mason 1991). La relation entre l'enrichissement et la stéréotypie n'est, cependant, pas encore très claire : ainsi, des études indiquent que l'enrichissement pourrait augmenter la motivation de souris à explorer, et cela pourrait être associé à la motivation à accroître l'aire vitale (Nevison *et al.* 1999b).

1.3.2 Stéréotypie et héritabilité

La stéréotypie relève, en fait, de multiples facteurs, dont l'effet n'est pas nécessairement additif (Würbel 2001). Des études qui se penchent sur son ontogenèse suggèrent qu'elle peut avoir une base génétique (Ödberg 1986). La propension à développer des comportements stéréotypiques peut être héritable, et son expression, liée à des stimuli stressants. Dans une

étude portant sur des campagnols roussâtres, *Clethrionomys glareolus,* Schoenecker et Heller (2000) montrent que la stéréotypie est approximativement sept fois plus fréquente chez la progéniture de parents stéréotypiques (même quand un seul des parents l'est) que chez celle de parents qui ne le sont pas. Schwaibold et Pillay (2001) ont aussi relevé que, chez des souris *Rhabdomys pumilio*, la stéréotypie est environ quatre fois plus commune chez la progéniture de femelles stéréotypiques que chez celle de femelles non stéréotypiques.

1.4. Objectifs de la maîtrise

Dans le chapitre qui suit, nous nous proposons d'abord d'étudier sur deux générations de souris sylvestres (*Peromyscus maniculatus sonoriensis*) sauvages maintenues en laboratoire l'effet de la consanguinité, de l'enrichissement (social et/ou physique) et de l'âge sur l'activité, puis de tester l'effet combiné de la consanguinité et de l'enrichissement (social et/ou physique) sur l'expression de la stéréotypie en intégrant l'activité dans le modèle afin de contrôler son effet sur la stéréotypie. Nous vérifions ensuite si les animaux varient entre eux dans leurs comportements (activité et stéréotypie), mais font preuve de constance individuelle au fil du temps.

CHAPITRE I : INBREEDING AND ENRICHMENT: EFFECTS ON STEREOTYPY IN CAPTIVE DEER MICE (*PEROMYSCUS MANICULATUS SONORIENSIS*)

2.1 Abstract

Inbreeding negatively affects fitness of individuals, and inbred animals are more likely to suffer from inbreeding depression, especially in harsh environmental conditions. Yet little is known about how inbreeding and environment interact to influence behaviour in the context of environmental stress such as poor welfare. Stereotypy is common in captive animals, particularly in those that are confined in physically and/or socially restricted environments. However, individuals may react differently to the same conditions, and they often differ in their propensity to show stereotypy. One explanation is that animals show consistent differences in behavioural traits of ecological importance such as activity, and that individuals differing in these behaviours are more or less sensitive to confined or socially restricted environments. In this study, we test if individual deer mice (*Peromyscus maniculatus sonoriensis*) differ in their stereotypic locomotor behaviours, and if these differences are related to temperament (i.e. activity), physical and/or social enrichment and inbreeding. We carried out daily 5-minute focal samples in the home cage of approximately 200 laboratory-bred deer mice from two generations, from age 20 days until 90 days, and used the method of instantaneous scan samples during 7-9 days, every half hour from 9:00 to 16:00, when they were aged between 1 and 1,5 years, in order to quantify stereotypic locomotor behaviours and activity levels. We ran mixed models on activity and stereotypy, with mouse identity nested in family as random effects. We then calculated repeatability, the proportion of the total variance due to the individual. We extracted the best linear unbiased predictors, which were used as the temperament and stereotypy values for each individual in correlation tests between juveniles and adults. Non-enriched G_1 juvenile inbreds exhibited more stress-related behaviour as indicated by stereotypy than enriched outbreds. However, this effect was not observed in G_1 adults and G_2 mice. Furthermore, poor social and/or physical conditions interacted with activity and increased the propensity to develop stereotypy. Differences in general activity and stereotypic behaviours were observed throughout the lifetime of individuals in relation to environmental (physical and social enrichment) or intrinsic (age, inbreeding) conditions. However, the same conditions had different effects with regards to age. Individuals from the same family resembled each other more than non-related individuals regarding stereotypy and activity; with time, intra-individual consistency increased. An understanding of the links between stereotypy and temperament should allow us to adaptively manage the differing needs of captive individuals.

Keywords: temperament, activity, stereotypy, enrichment, inbreeding, deer mice.

2.2 Introduction

Inbreeding is an increasing source of concern in wild, domestic and laboratory animals; it is widespread, and can have strong implications for conservation and for welfare of captive-bred animals (Charlesworth & Charlesworth 1987; Ralls et al. 1988; Thornhill 1993; Bijlsma et al. 1997). Furthermore, not taking its effect into account may bias the results of research using animal models. Inbred animals are more likely to be homozygous for recessive lethal or deleterious alleles, and to suffer from inbreeding depression, defined as a reduction of the mean phenotypic value of a trait due to inbreeding (Falconer & Mackay 1996). A growing number of studies have documented inbreeding depression in wild populations (reviewed in: Crnokrak & Roff 1999; Frankham et al. 2002; Keller and Waller 2002) and in domesticated animals (Wiener et al. 1992; Smith et al. 1998). Overall, inbreeding affects the fitness of individuals through life history traits such as reproduction, survival, offspring size, number and survival, body size physical abnormalities, and resistance to illness (Charlesworth & Charlesworth 1987; Margulis & Altmann 1997; Margulis 1998; Roff 1998). Most studies assess some of its short-term effects, particularly those appearing at birth. Nevertheless, inbred individuals that survive to adulthood may express unfavourable phenotypes in the later stages of life (Sharp 1984; Charlesworth & Charlesworth 1987; Margulis & Altmann 1997). For instance, their reproduction may be reduced due to physiological deficiencies or altered behaviours. They may be unable to raise their progeny, and their offspring, though not necessarily inbred themselves, may have a reduced fitness.

Inbreeding does not affect all traits to the same degree: those closely associated with fitness (e.g. viability, fertility and disease resistance) are more prone to inbreeding depression than morphological traits (Clayton et al. 1957; Falconer and Mackay 1996; DeRose and Roff 1999). Inbreeding can also have an impact on behaviour (Meffert et al. 2002). For example, inbreeding has been observed to affect parent-offspring interactions, maternal care (Margulis, 1997), activity levels following pairing (Margulis & Altmann 1997; Margulis 1998; Meffert, Hicks & Regan 2002), mating display and male sexual motivation (Aspi 2002; Mariette et al. 2006). Furthermore, inbreeding depression may be exacerbated by novel or harsh environmental conditions (Frankham 1995; Falconer & Mackay 1996; Roff 1998; Bijlsma et al. 1999; Meagher et al. 2000; Frankham et al. 2002; Joron and Brakefield 2003;

Kristensen et al. 2003; Reed et al. 2003), indicating that inbreeding can reduce the capacity to face environmental stress (Jimenez et al. 1994; Miller 1994; Armbruster & Reed 2005). One reason for this is that deleterious alleles that are silent under benign conditions can be expressed under novel or stressful conditions (Rutherford & Lindquist 1998; Bijlsma et al. 2000; Queitsch et al. 2002; Vermuelen & Bijlsma 2004).

To our knowledge little is known about how environment and inbreeding interact to influence behaviour, particularly in the context of environmental stress such as poor welfare. Yet livestock or laboratory animals are often housed at low costs and exposed to overcrowding or emotional deprivation, and restrictive physical conditions. Inbred individuals may be particularly sensitive to the sources of stress associated with captivity. In this paper, we test the hypothesis that enriching the environment can decrease inbreeding depression on behavioural traits in captive animals. We chose to observe abnormal repetitive behaviour — traditionally called stereotypy — in captive bred deer mice, *Peromyscus maniculatus sonoriensis*. If enrichment, whether social, physical, or both, interacts with inbreeding in such a way that it reduces the effect of deleterious genes, it could substantially reduce the chances of developing stereotypic behaviours in captivity. Stereotypy is a common behaviour in many captive animals raised in non-naturalistic environments. It is characterized by a relatively invariant pattern, regular repetition and apparent uselessness (Fox 1965). Stereotypies are often linked to past or ongoing stress partly due to environmental features, and many researchers have centred their attention on such aspects as confined enclosures, barren and restrictive conditions, non-stimulating environments and social deprivation, i.e. on standard *versus* enriched conditions (Dantzer 1986; Odberg 1987). The standard definition of an environmental enrichment (EE) is a 'combination of complex inanimate and social stimulation' (Rosenzweig et al. 1978). Some studies show that the frequency of stereotypic behaviour can decrease when the size and the complexity of a captive animal's environment is increased (Fraser 1975; Redbo 1990; both cited in Powell et al. 2000). For instance, in a study involving deer mice, it was noted that standard-caged individuals engaged in stereotypic behaviours at a higher rate and developed these behaviours more quickly than animals in enriched cages (Powell et al. 1999). Rodents reared in standard cages suffer from impaired brain development, stereotypies and an anxious behavioural profile when compared to enriched

animals (see review in Van Praag et al. 2000; Würbel 2001; Wolfer et al. 2004). Conversely, an enriched environment induces a host of structural changes in the brains of rodents (see review in Van Praag et al. 2000). Also, C3H mice, *Mus musculus*, raised in an enriched environment show decreased fearfulness and anxiety-like behaviour, as well as a reduced response to stress compared to control mice (Benaroya-Milshtein et al. 2004). Social isolation can also have negative effects and lead to behavioural abnormalities (Broom 1981): for example, nonhuman captive primates that experience early maternal or social deprivation develop stereotypies like body rocking and tail biting (Harlow et al. 1965, cited in Powell et al. 1999).

Individuals of a same species vary in their sensitivity to environmental conditions, and they often differ in their propensity to develop stereotypies; once they do develop these behaviours, they tend to be consistent over time, at least as long as the environment remains unchanged (see review by Mason 1991). There are often underlying physiological bases to this variation among individuals, but the reasons of the latter are still not completely understood. One explanation is depending on their temperament (*sensu* Réale et al. 2007: consistency of individual behavioural differences over time and/or across situations) individual animals are more or less sensitive to physically or socially restricted environments. Animals with different personalities may react differently to the stress of captivity (McDougall et al. 2006). Inter-individual, inter-strain and inter-specific differences in the form and level of stereotypy performance do exist (Würbel & Stauffacher 1994, cited in Würbel 2006; Nevison et al. 1999b), and some researchers have concluded that stereotypy may be related to differences in general activity. For instance, individual levels of stereotypy and activity within populations of bank voles, *Clethrionomys glareolus*, are correlated (Ödberg 1986). Bank vole pups that had developed stereotypic locomotor behaviour by 60 days of age in their home-cage spent more time on non-repetitive locomotor activities at 10 days of age than non-stereotypic voles (Cooper & Nicol 1996). Other studies support the suggestion that, within a population, the most stereotypic individuals are also the most active (e.g. Bildsoe et al. 1991).

The overall aim of this study is to test in the laboratory whether environmental enrichment (social and/or physical) decreases the effects of inbreeding on stereotypic behaviours using individual deer mice as a model and taking into account a trait of temperament (general

activity). We observed mice in their home cage from age 20-90 days and from 1-2 years. We used mixed models to assess effects of enrichment and inbreeding on a temperament trait (activity) in mice, both as juveniles and as adults, from two successive generations. We also used mixed models to estimate effects of enrichment, inbreeding and temperament on stereotypic behaviours of the same mice. We then estimated inter-individual and inter-family variation and we tested whether deer mice showed consistent differences over time in activity levels and stereotypic behaviours. We extracted from the mixed linear models individual behavioural profile values for activity and stereotypy. Using these values we examined stereotypic and activity correlations between juvenile and adult stages.

2.3. Material and methods

2.3.1 Study subjects

The subjects for the current study are laboratory-bred deer mice, *Peromyscus maniculatus sonoriensis*. The ancestral population was derived from a wild-caught stock captured in California in 1995 (50 initial individuals) and bred by the Peromyscus Genetic Stock Center (PGSC). Breeding conditions have been controlled by the PGSC in order to keep inbreeding coefficients, F, as low as possible. Sixty-four individuals from 12 families (referred to thereafter as Generation 0, G_0,), with a known pedigree, were the founders in 2004 of the next two generations (referred to as G_1 and G_2 thereafter) bred in our animal care facility.

Mice were housed in either a standard or a physically enriched environment. The standard environment consisted of a plexiglas cage (Animal Care Systems Inc, 30.5 cm x 15 cm x 18 cm — L x W x H) in G_1 mice from age 0 to age 90 days. It included a central food hopper, a bottle of water and two pieces of cotton. Older G_1 mice and all G_2 individuals were housed in plexiglas cages (Lab Products Inc, 30.5 cm x 15 cm x 18 cm) containing a peripheral food hopper, a bottle of water, two pieces of cotton and 1 or 2 black rubber joints to satisfy welfare requirements. The enriched environment consisted of a plexiglas transparent cage (Lab Products Inc, 47.6 cm x 35.2 cm x 19.7 cm) with an inner wire mesh lid, two water bottles, and a food hopper. The enrichment elements included one

or two plastic igloos with running wheels, several orange-tinted plastic pipes and black rubber joints. The spatial arrangement of the igloos and of the pipes was modified every week. In both housing conditions, the base of the cage was covered with wood shavings, and the animals had *ad libitum* access to water and food (Charles River rodent ; Agribands Canada). Two compressed pieces of cotton were also provided weekly as nesting material. Once a week, the cages were cleaned and supplied with fresh litter; water bottles were replaced and food hoppers were replenished. Mice were kept in two rooms at 22-24°C and an inverse 14:10LD photoperiod.

2.3.2 Breeding procedure

To create families, we placed a male and a female together in a cage until we could detect the first signs of pregnancy. We then transferred the male to another cage to minimize the danger of exposing newborns to male aggressive behaviour and to limit potential fertilization of the female right after birth. When the females' pregnancy went unnoticed, the pair was separated right after the birth of the first litter and in some cases a second litter was produced. Twelve-day old pups were individually marked with ear-punches with a predetermined code. Pups remained with their mother until their weaning, at 31 days of age. The mother was then put again with her sisters, and her litter was divided in groups of siblings according to their sex (no litter was culled).

We assigned the G_0 stock mice and all of their descendants to either the enriched environment or the standard environment for the duration of the study. Inbreeding coefficient at G_0 averaged 0.048 (range 0-0.17; see Hartl & Clark 1989 for details on inbreeding coefficient). We paired 36 G_0 full-sibs and 34 non-related individuals to produce inbred and outbred G_1 offspring, respectively. Mice were never mated before 60 days of age. After, four months, 28 pairs had reproduced (see Table 2.1 for details on number of families and pups at each generation).

Table 2.1

G_1 and G_2 experimental design and sample sizes for the *Peromyscus maniculatus* captive-bred population. *F* = inbreeding coefficient; I = inbreds (F ≥ 0.25); O = outbreds; S = standard environment; E = enriched environment. Numbers in parentheses refer to the mice observed for the study on the ontogeny of stereotypy

Generation	Enrichment	Inbreeding	Nb of individuals	Nb of families	F
G_1	S	Outbred	27 (18)	6 (4)	0.04-0.07
	S	Inbred	26 (13)	7 (3)	0.26-0.29
	E	Outbred	37 (20)	7 (5)	0.02-0.06
	E	Inbred	37 (28)	8 (6)	0.25-0.33
G_2	S	Outbred	29 (20)	4 (4)	0.05-0.10
	S	Inbred	9 (9)	2 (2)	0.27-0.28
	E	Outbred	45 (33)	7 (7)	0.06-0.18
	E	Inbred	52 (47)	7 (7)	0.27-0.29

Later, we produced a second generation (G_2) by pairing 72 G_1 mice according to their inbreeding coefficient and their environment type (Table 2.2). In this set-up, to obtain offspring with a wider and more continuous range of F values without increasing F beyond 0.33 (approximately the F of offspring born from a brother-sister mating), we crossed outbreds with outbreds, cousins with cousins, inbreds with outbreds, or inbreds with inbreds from different families. Only 58 % of the couples had reproduced after four months.

Table 2.2

Pairing scheme of G_1 and G_2 based on environment type, maternal inbreeding, paternal inbreeding and inbreeding of potential future litters (inbred = F ≥ 0.25)

Inbreeding status			**Standard environment**		**Enriched environment**	
Maternal	**Paternal**	**Litter**	Couples	Couples which reproduced	Couples	Couples which reproduced
G_1						
O	O	I	9	7	9	8
O	O	O	8	6	9	7
G_2						
I	I	O	4	1	5	2
O	O	O	3	3	5	5
O	O	I	5	2	8	7
O	I	O	1	0	3	1
I	O	O	1	0	1	0

I = inbreds; O = outbreds; G = generation.

In both types of environments, increasing the number of conspecifics up to a certain point per

cage constitutes a social enrichment. In this study, the number of individuals per cage varied between 1 and 6 following weaning.

2.3.3 Behavioural observations of the level of stereotypy and activity in the home cage

In order to limit disturbances, mice were observed in their home cage under reduced light. Prior to the study, we observed G_0 mature individuals for a period of 2 months in order to define a set of typical repetitive locomotor behaviours. The stereotypies were defined as follows: vertical jumping; lateral jumping; somersaulting; jumping on a wire mesh; patterned running; "straightening up" movements which may or may not be followed by lateral running; circling (i.e. running in tight circles as after its own tail); digging on cage walls or floor. We did not take bar-chewing into account, because it was difficult to tell if mice were gnawing on bars or eating. We considered a series of at least three repetitions of one of the behaviours listed above a stereotypic bout. Two series separated by at least 5 seconds were considered as two different stereotypic bouts.

We collected data between 8:00 and 16:00, a period encompassing the majority of the animals' active period. During the recording sessions, for G_1 mice between ages 20 and 90 days, we placed a dim yellow light (40 Watts) at half a meter from the home cages. We did all other observations in the home cage in the dark, using an infrared camera to record the movements of the focal mouse. We observed one family at a time; the order of observation of individuals was randomized. We ran 5 min/day focal observations on pups. The onset of stereotypy development in *Peromyscus* is reported to be at around 20 days of age in mice weaned at 3 weeks, and stereotypies are supposed to be fully established at maturity (i.e. 8 weeks in deer mice) (see review by Mason 1991). We thus observed each mouse 5 minutes every day between 20 and 31 days of age, then every other day up to 45 days, then at 55, 60, 75 and 90 days. During the 5 min observation of stereotypic behaviours, we also estimated the level of activity of the mice in their home cage. During each focal observation, animals were categorized as either a) non-active (immobile with eyes open or closed $\geq$ 65 % of the time) or b) active (eating, drinking, grooming or moving $\geq$ 65 % of the time) (around 23 observations per mice).

To assess the daily levels of activity and stereotypy at an adult stage, we ran instantaneous scan samples on individual mice between ages 1 and 2 years, over 6-9 days. Observations were done every 30 min between 9:00 and 16:00 (between 90 and 135 observations per mouse). During scans, each individual was noted as not active (score = 0; lying with eyes open or closed) or active (score = 1; eating, running on a wheel, grooming, sniffing/exploring cage, burrowing under the wood-shavings, stereotyping...), whereas stereotypic behaviour was determined with a presence (score = 1) or absence (score = 0) scale.

2.3.4 Statistical analyses

We assessed repeatability of stereotypic behaviours and activity by replicating measures of these behavioural traits. Frequency of stereotypic behaviours depends on the length of a stereotypic bout, which itself depends on the type of stereotypic behaviour performed by the mouse. For instance, it takes more time to do one patterned run along the walls of the cage than to do one jump. Furthermore, frequency of stereotypic behaviour depends on the level of activity (some animals that are not very active spend most of their active time performing stereotypic behaviours, while some very active individuals spend a small proportion of their active time engaging in stereotypy). Thus, for each focal observation (i.e. juvenile stage), we coded the animal as stereotypic (i.e. showing at least one stereotypic bout: score = 1) or not (i.e. score = 0). Observations on juveniles were grouped in three categories (category 1, before weaning = 20 to 31 days of age; category 2 = 33 to 45 days of age; category 3, mature pups = 55 to 90 days of age). For each age class category, we calculated the proportion of focal observations for which an individual was "stereotypic" or "active". For mice aged between 1 and 2 years, we calculated the daily proportion of scan observations for which the mouse was "stereotypic" or "active", respectively. We used these proportions as the dependent variables for further analyses.

Prior to analyses using linear mixed models, proportion of focal/scan spent stereotyping or being active were arcsine transformed. We performed all analyses using R (version 2.6.0). We analysed juveniles and adults, and G_1 and G_2, separately, running linear mixed models to avoid problems of non-independence of the data (caused by repeated measures for each

mouse, and the pedigree structure of our samples of mice; Pinheiro and Bates 2002). For each model, we nested mouse identity within family identity as random effects. This procedure allowed us to predict the variance of each random effect as a proportion of the total variance of random effects and to calculate repeatability, the proportion of the total variance due to the individual (Lessells & Boags 1987). Mixed models also allowed us to estimate the fixed effects on stereotypic behaviour or activity independently of individual or family effects, including maternal effects.

Inbreeding, age, number of individuals per cage, type of environment and relevant first order interactions (Inbreeding:Cage, Inbreeding:Number of individuals, and Cage:Number of individuals, and Inbreeding:Age in juveniles) were treated in the activity model as fixed effects. Activity, inbreeding, number of individuals per cage, type of environment and relevant first order interactions (Inbreeding:Cage, Inbreeding:Number of individuals, Inbreeding:Activity, Cage:Number of individuals, Number of individuals:Activity and Cage:Activity, and Inbreeding:Age in juveniles) were treated in the stereotypy model as fixed effects. To test the fixed effects, we first ran a model including all the fixed effects terms and the relevant two-way interactions. The significance of interactions was then examined, before using stepwise backward elimination of the fixed effects (removing the least significant first order interaction and rerunning the model, than repeating the process until the least significant fixed effect was excluded; significance for interactions and main effects was set at $p = 0.05$ using F-values). The selected model only included the significant main terms as well as interactions and non-significant terms involved in a significant interaction (Pinheiro and Bates 2000). We tested the significance of random effects by comparing three models (model 1 = with family and mouse ID; model 2 = with family ID; model 3 = with mouse ID) using a log-likelihood ratio test (i.e. LLR = 2 (loglikelihood of model 1 – loglikelhood of model 2). Comparing model 1 with 2 and 3 provides a test of significance for individual and family effects, respectively. LLR tests were done while keeping the fixed effect structure unchanged (Pinheiro and Bates 2000). All the analyses of G_1 adults were first done on the whole G_1 adult database, followed by separate tests using a G_1 adult database excluding all individuals that had not been observed as juveniles. We obtained similar results and, therefore, only the first series of tests will be shown here.

2.3.5 Correlation between individual behaviour as juveniles and adults within a generation

To assess correlations between juvenile and adult mice for stereotypic behaviours and activity, we extracted the best linear unbiased predictors (BLUPs) for random effects from the models containing only the significant effects. BLUPS are predictors of individual behaviour profiles independent of fixed effects and are less sensitive to extreme values within data than separate regression estimates (Pinheiro & Bates 2000). They provide better estimates of the behavioural profile of an individual than the mean of all the measures for that individual (Martin & Réale 2008).

2.4 Results

2.4.1 Activity

In both G_1 and G_2 juveniles, activity level increased with age. An interaction between inbreeding and number of individuals significantly affected activity such that at a low number of individuals, inbreds were more active than outbreds, while the reverse was observed at a higher number of individuals (Table 2.3, Fig. 2.1). No other effects were significant. In adults, activity increased with number of individuals in G_1 but decreased in G_2 mice (Table 2.3). We could not find any other significant effects on activity.

Variance in activity caused by family was significantly different from zero in G_1 and G_2 models for juveniles and adults; variance in activity caused by identity was significantly different from zero in G_1 and G_2 models for adults only (Table 2.3). In juveniles, repeatability of activity [$r = (V_{family} + V_{individual}) / (V_{family} + V_{individual} + V_{residual})$] was low, ranging from 0.08 to 0.23, while in adult mice it was higher ($r = 0.64$). In G_2 mice, a significant positive correlation was observed between BLUP values of juveniles and BLUP values of mature individuals for activity ($r = 0.47$, $t = 5.63$, $df = 112$, $p < 0.01$); but not in G_1 mice (Fig 2.2).

Table 2.3

Estimates of random and fixed effects produced by the minimally adequate linear mixed model of activity in generation 1 and generation 2 *Peromyscus maniculatus*. Individual was nested within family as random effects. Initial fixed effects included inbreeding (I), cage type (C), number of individuals per cage (NB), and age group (A) in juveniles, and relevant first order interactions: IxC, IxNB, and CxNB, and IxA in juveniles.

Juveniles (20-75 days of age)

Random effects	**Generation 1**					**Generation 2**				
Family variance†	**8.14 %**					**16.12 %**				
Individual variance	0 %					7.53 %				
Residual variance	91.86 %					76.33 %				
Fixed effects	Val.	Std. E	df	t-val.	P-val	Val.	Std. E	df	t-val.	P-val.
(Intercept)	1.00	0.05	150	17.42	0.00	1.01	0.06	212	15.78	0.00
I	-0.03	0.05	16	-0,69	0.49	-0.05	0.07	18	-0.70	0.48
NB	-0.07	0.02	150	-3,14	**0.00**	0.00	0.01	212	0.00	0.99
A2	0.08	0.07	150	1.06	0.28	0.27	0.05	212	4.92	**0.00**
A3	0.24	0.07	150	3.16	**0.00**	0.35	0.05	212	6.52	**0.00**
I×NB	0.04	0.02	150	2.29	**0.02**	0.06	0.02	212	2.19	**0.02**

Mature (1-2 years)

Random effects	**Generation 1**					**Generation 2**				
Family variance	**12.43 %**					**23.52 %**				
Individual variance	**51.79 %**					**41.10 %**				
Residual variance	35.76 %					35.37 %				
Fixed effects	Val.	Std. E	df	t-val.	P-val	Val.	Std. E	df	t-val.	P-val.
(Intercept)	0.90	0.03	588	26.21	0.00	1.11	0.04	786	25.39	0.00
NB	0.05	0.02	82	2.30	**0.02**	-0.05	0.01	113	-3.15	**0.00**

† Variance components are expressed in percent of the total variance.
Significant random effects, based on log-likelihood tests, are in bold.
*Non significant fixed effects were removed during model selection with $P > 0.05$ for simple effects and interactions using t-values.
A1=20-31 days of age; A2=33-45 days of age; A3=55-90 days of age.
Inbreds are considered as the reference for inbreeding in the model.
Enriched is considered as the reference for environment in the model.
Age1 is considered as the reference for age category in the model.

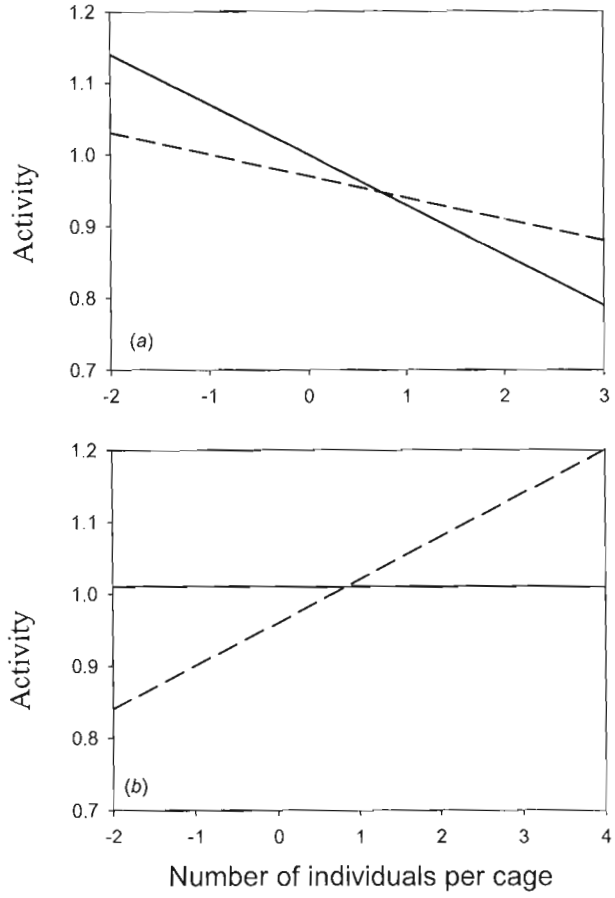

Figure 2.1 Combined effects of inbreeding and number of individuals per cage on (a) G_1 juvenile and (b) G_2 juvenile deer mice activity (see Table 2.4 for details on the linear mixed model). Inbred mice are represented with the solid lines, and outbred mice are represented with the dashed lines. Stereotypy and activity have been arcsine transformed, and number of individuals has been standardized according to the mean *(-2=1 individual per cage; -1=2 individuals; 0=3 individuals, 1=4 individuals; 2= 5 individuals; 3=6 individuals; 4= 7 individuals).*

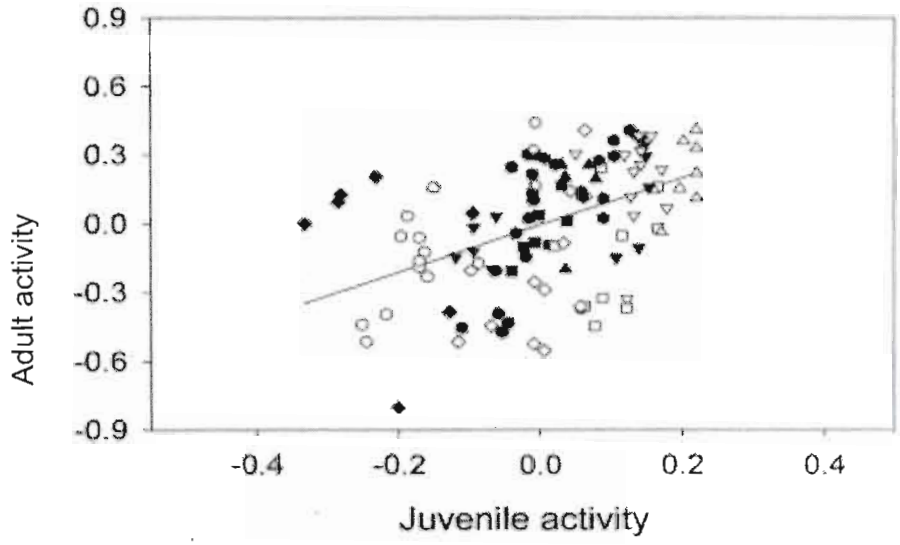

Figure 2.2 Correlations between G_2 behaviours as juveniles and as adults. Each dot represents an individual and each symbol represents a family. Activity has been arcsine transformed and standardized according to the mean.

2.4.2 Stereotypy

Inbred G_1 juvenile mice developed significantly more stereotypic behaviours in a standard than in an enriched environment, while this did not hold true for outbreds (significant interaction between inbreeding and cage type; Table 2.4, Fig. 2.3). This effect varied with age and generation: it neither affected G_1 nor G_2 adult mice.

In both G_1 and G_2 pups, stereotypy of very active animals housed in physically or socially enriched cages was negligible in comparison with standard-raised mice or socially deprived mice; the effect of physical enrichment was still observed in mature mice. That is, in G_1 and G_2 juveniles, stereotypy increased with an increasing activity and a decreasing number of individuals (Table 2.4, Fig. 2.4), but no significant effect was observed in G_1 and G_2 mature individuals (Table 2.4). Stereotypy also increased more steeply and reached higher levels with activity in standard cages than in enriched cages in G_1 and G_2 pups, and both G_1 and G_2 mature individuals (Table 2.5, Fig. 2.5). In G_2 mature mice, activity also affected stereotypy through an interaction with inbreeding, such that stereotypy decreased with activity in inbreds while it increased in outbreds (Table 2.4, Fig. 2.6). No other significant effects were observed.

Variance in the proportion of time spent "stereotyping" caused by family and identity were significantly different from zero in both G_1 and G_2 juvenile and adult mice (Table 2.4). Stereotypy showed repeatabilities of 0.37 and 0.35 for juveniles of G_1 and G_2, respectively, and of 0.77 and 0.64 for adults of G_1 and G_2, respectively. Significant positive correlations were observed between BLUP values of juveniles and BLUP values of mature mice for stereotypy in G_1 ($r = 0.24$, $t = 2.00$, $df = 65$, $p = 0.04$) and G_2 mice. ($r = 0.53$, $t = 6.71$, $df = 112$, $p < 0.0001$) (Fig. 2.7 a and b). For both generations individual variance in stereotypy increased, whereas family variance decreased with age.

Table 2.4

Estimates of random and fixed effects produced by the minimally adequate linear mixed model of stereotypy in generation 1 and generation 2 *Peromyscus maniculatus*. Individual was nested within the family as random effects. Initial fixed effects included inbreeding (I), cage type (C), number of individuals per cage (NB), activity (Ac), and age group (A) in juveniles, and relevant first order interactions: IxC, IxNB, IxAc, CxNB, NBxAc and CxAc, and IxA in juveniles.

Juveniles (20-90 days of age)										
Random effects	**Generation 1**					**Generation 2**				
Family variance	**23.86 %**					**14.33 %**				
Individual variance	**13.33 %**					**20.93 %**				
Residual variance	62.79					64.72 %				
Fixed effects	Val.	Std.E	df	t-val.	P-val	Val.	Std. E	df	t-val.	P-val.
(Intercept)	0.14	0.07	150	1.78	0.07	0.25	0.07	210	3.47	0.00
I	0.13	0.11	14	1.12	0.28	-	-	-	-	**na**
C	0.56	0.13	14	4.09	**0.00**	0.30	0.10	18	2.90	**0.00**
NB	-0.04	0.01	150	-2.77	**0.00**	-0.02	0.02	210	-1.13	0.25
Ac	0.17	0.08	150	2.05	**0.04**	0.38	0.07	210	5.11	**0.00**
A2	-	-	-	-	**na**	0.17	0.07	210	2.45	**0.01**
A3	-	-	-	-	**na**	0.41	0.07	210	5.59	**0.00**
I×C	-0.65	0.18	14	-3.47	**0.00**	-	-	-	-	**na**
C×Ac	0.40	0.12	150	3.31	**0.00**	0.33	0.14	210	2.41	**0.01**
NB×Ac	-0.07	0.03	150	-2.02	**0.04**	-0.11	0.04	210	-2.57	**0.01**

Mature (1-2 years)										
Random effects	**Generation 1**					**Generation 2**				
Family variance	6.71 %					**9.03 %**				
Individual variance	**71.22 %**					**55.04 %**				
Residual variance	22.06 %					35.92 %				
Fixed effects	Val.	Std.E	df	t-val.	P-val	Val.	Std.E	df	t-val.	P-val.
(Intercept)	0.27	0.04	586	6.56	**0.00**	0.57	0.05	783	11.27	0.00
I	-	-	-	-	**na**	0.05	0.06	17	0.83	0.41
C	0.16	0.06	26	2.47	**0.02**	0.15	0.07	17	2.05	**0.05**
Ac	0.17	0.03	586	4.89	**0.00**	0.41	0.05	783	8.09	**0.00**
I×Ac	-	-	-	-	**na**	0.23	0.06	783	3.40	**0.00**
C×Ac	0.52	0.06	586	8.08	**0.00**	0.14	0.07	783	1.98	**0.04**

† Variance components are expressed in percent of the total variance.

Significant random effects, based on log-likelihood tests, are in bold.

*Non significant fixed effects were removed during model selection with $P > 0.05$ for simple effects and interactions using F-values.

Where na is indicated as a P-value, the fixed effect was not retained in the final linear mixed model.

A1=20-31 days of age; A2=33-45 days of age; A3=55-90 days of age.

Inbreds are considered as the reference for inbreeding in the model.

Enriched is considered as the reference for environment in the model.

Age1 is considered as the reference for age category in the model.

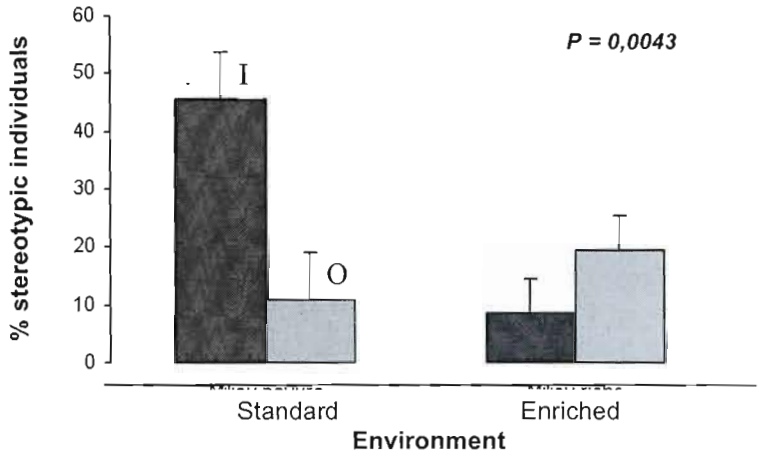

Figure 2.3 Combined effects of inbreeding and cage type on G_1 juvenile deer mouse stereotypy (see Table 5 for details on the linear mixed model). Graph was drawn using raw data. I=inbred, O = outbred.

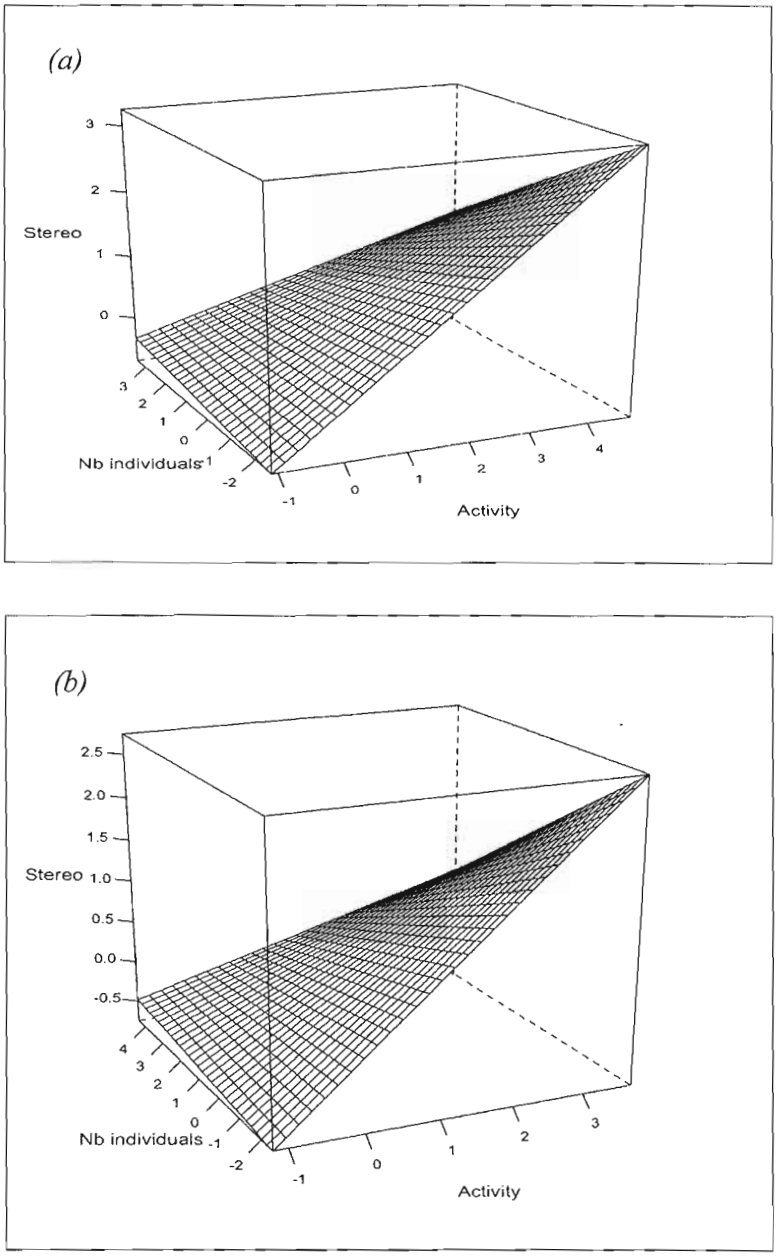

Figure 2.4 Combined effects of number of individuals per cage and activity on stereotypic behaviours of G_1 juvenile deer mice (a) and G_2 juvenile deer mice (b) (see Table 2.4 for details on the linear mixed model). Stereotypy and activity have been arcsine transformed, and both activity and number of individuals have been standardized according to the mean *(-2=1 individual per cage; -1=2 individuals; 0=3 individuals, 1=4 individuals; 2= 5 individuals; 3=6 individuals; 4= 7 individuals).*

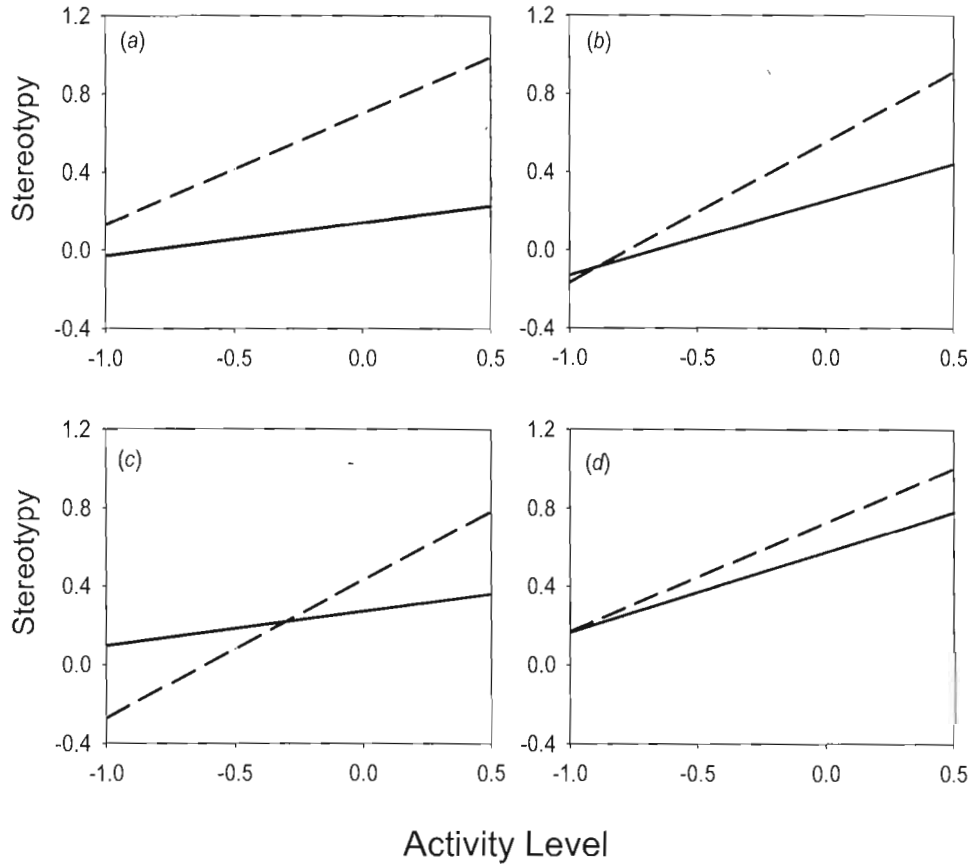

Figure 2.5 Combined effects of activity and cage type on stereotypic behaviours of (a) G_1 juveniles, (b) G_2 juveniles, (c) G_1 mature individuals, and (d) G_2 mature individuals (see Table 2.4 for details on the linear mixed model). Solid lines represent mice from enriched cages and dotted lines represent mice from standard cages. Stereotypy and activity have been arcsine transformed, and activity has been standardized according to the mean.

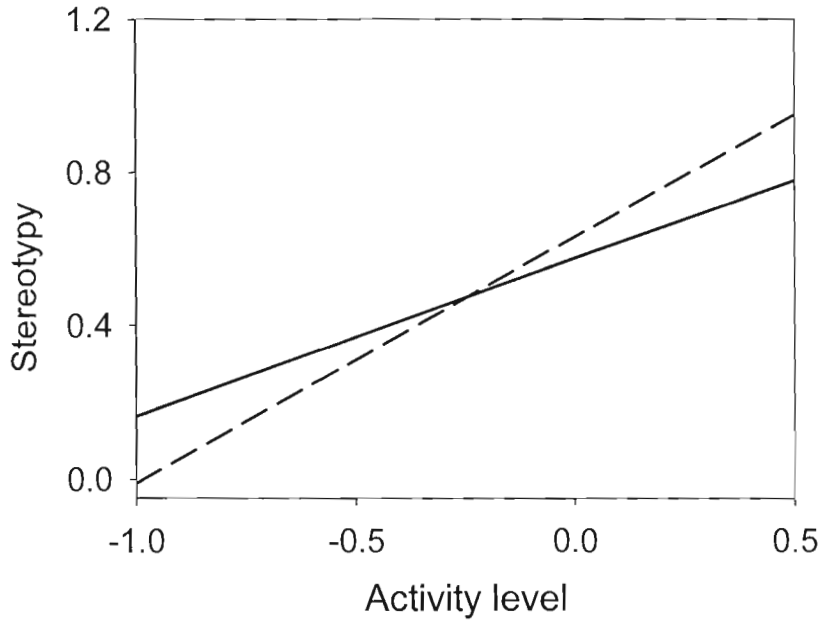

Figure 2.6 Combined effects of inbreeding and activity on G_2 adult deer mice stereotypy (see Table 2.4 for details on the linear mixed model). Stereotypy and activity have been arcsine transformed, and activity has also been standardized according to the mean.

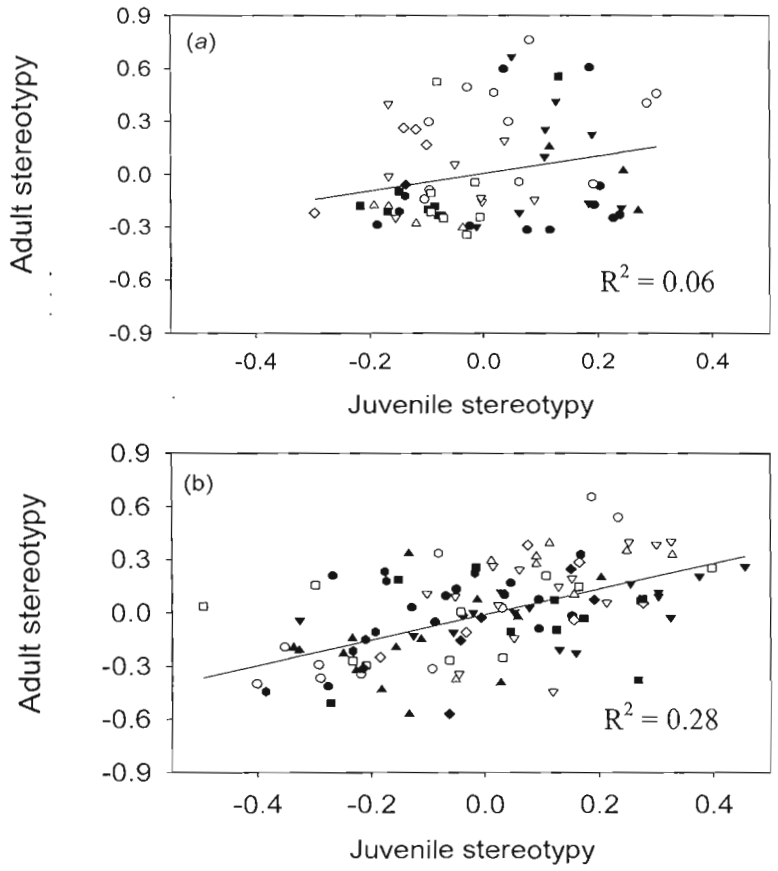

Figure 2.7 Correlations between mice stereotypic behaviours as juveniles and as adults: a) G_1 stereotypy and b) G_2 stereotypy. Each dot represents an individual and each symbol represents a family. Stereotypy and activity have been arcsine transformed and standardized according to the mean

2.5 Discussion

2.5.1 Effects of inbreeding, and its interaction with enrichment on stereotypy or activity

According to our expectations, inbred G_1 juveniles developed significantly more stereotypic behaviours in the standard than in the physically enriched environment, while this did not hold true for outbreds (Fig. 2.3). In other words, the standard environment was detrimental to young inbreds, and the enriched condition greatly reduced their propensity towards stereotypy, while physical enrichment had little effect on the outbreds' stereotypic behaviours. However, this study shows us that there is no simple answer concerning the effects of inbreeding and physical enrichment on stereotypy: 1) we expected this effect to be maintained in adult G_1 inbred mice, and furthermore 2) to be observed in inbred mice irrespective of generations, which was not the case. Inbreeding effects in G_1 juveniles seem to differ from those observed at a later stage of development inasmuch as inbreeding does not have a permanent impact on stereotypy. Inbreeding does not influence G_1 adults, which might suggest that apart from being trait specific, it affects traits differently across life stages (Ober et al 1999; Margulis 1998). For instance, captive populations of Mexican and red wolves do not exhibit inbreeding depression for juvenile survival (Kalinowski et al. 1999), but do show inbreeding depression for adult survival (Wilcken 2001, in Breitenmoser-Würsten 2007). Different genes affect traits at different stages in development (Vermeulen & Bijlsma 2004), therefore inbreeding depression might be expressed differently depending on which loci are homozygous. Our results suggest that the deleterious effects of inbreeding on stereotypy genesis and maintenance may be transient. Another possible explanation for the difference observed within and between generations is that the standard cages, once equipped with a rubber pipe providing shelter, may have become a mild source of environmental stress compared with enriched cages: the physically very small change in the environmental conditions of standard cages (the addition of a shelter for welfare reasons) may have had a significant biological impact on mouse behaviour notwithstanding the fact that G_1 mice were over 120 days of age when they benefitted from the pipe. Questioning the existence of a sensitive age relative to the genesis of stereotypic behaviours, researchers like Ödberg (1987) and Powell et al. (2000) found that in certain species, like bank voles (*Clethrionomys*

glareolus) and deer mice (*Peromyscus maniculatus*), improving the environment early in the post-weaning period or after 60 days of age inhibits or reduces frequency of stereotypic behaviours in most stereotypic animals, which supports the importance of environmental restriction in the development of stereotypy and the possible efficacy of enrichment in the prevention of this problem. However, different studies show that exposure to an enriched environment at different points in development has differential effects on stereotypy and is species-dependant (Powell et al. 1999, 2000; Hadley et al. 2006). For example, Cooper et al. (1996) found that when young bank voles were placed in an enriched environment, stereotypies ceased, whereas older animals placed in the same conditions continued to engage in stereotypic behaviour. If the addition of the rubber pipe in this study is involved in the change of stereotypic levels of inbreds, it supports the idea that young inbred deer mice are particularly sensitive to conditions eliciting stereotypies (such as a very poor physical environment lacking a shelter and species-specific requirements), and that an early post-weaning enrichment may reduce their stress enough to lower their level of stereotypic behaviours.

One could argue that another explanation for the disparity between generations of inbred mice could be accounted for by genetic factors, such as an increased randomness due to inbreeding. It has been documented that inbreeding reapportions the variance so that as families become more inbred, genetic variance between families increases, and genetic variance within families decreases (Falconer 1981; Hartl and Clark 1989). Some families will react more to an environmental stress than average, and some will react worse. All inbred mice in this study were produced by full-sib pairing. It seems doubtful that in our random creation of inbred families, we produced a generation of individuals that were more tolerant to stress as indicated by stereotypy, or that variation between inbreds was great, which buffered inbreeding effects. In other words, our results could hardly be attributed to chance differences in genetic makeup of individuals. On the contrary, there might have been no clear cut-off between inbreds and outbreds, especially in G_2 mice, which had higher inbreeding coefficients than G_1; it may be that the 'low' level of inbreeding of the 'outbred' mice (ranging between 0.02 and 0.18, given the difficulty in completely avoiding inbreeding in laboratory-reproduced animals) was enough to lead to some inbreeding depression. This may

be the reason why G_1 inbreds differ from outbreds but G_2 do not. In brief, the fact that inbreeding affected stereotypic behaviours of G_1 juveniles through an interaction with physical enrichment indicates that it can have a detrimental impact on stereotypy. But for its effects to be enhanced, the level of environmental stress has to be large enough to impact behaviour.

We also expected socially deprived inbred mice to develop more stereotypical behaviours, because deer mice are social animals; however, this was not the case. There was, though, a significant interaction between inbreeding and social enrichment on general activity in the home cage of juvenile mice of both generations — but not on adults — such that inbred juveniles were more active than outbreds at a low number of individuals per cage, while the reverse was observed with an increasing number of individuals (Fig. 2.1). The finding of a significant inbreeding × social enrichment interaction only among juvenile mice suggests that inbreeding effects on general activity are temporary, and may be lost later in development, as seen with the inbreeding × physical enrichment interaction in G_1.There was also an interaction between inbreeding and activity on stereotypic behaviours of G_2 adult mice such that active inbreds showed less stereotypy than active outbreds (Fig. 2.6). Although this result may seem counter-intuitive, our index of activity level included all behaviours that involved movement. Discerning between different types of activities, particularly those that require very different levels of energy expenditure (such as feeding versus running), may have yielded different results. If inbreeding influences the senescence rates in mice or deleterious genes expressed at later stages in life, we may expect inbred and outbred mice to differ in their use of energetically expensive activities. We found that inbred mice had much lower body mass that outbred mice (unpublished data), which may be indicative of higher senescence rates in the inbred strains (mutation accumulation hypothesis). Research relating inbreeding and behaviour is very sparse. In her study on the relationship between inbreeding and general activity during pairing, Margulis (1997) found that the inbreeding coefficient of a subspecies of female oldfield mice (*Peromyscus polionotus*) was a significant predictor of activity levels, with inbred mice less active than outbred ones. However activity levels were only measured in mature mice and during pairing, which does not give any indication on the relationship between inbreeding, social enrichment

and activity. Furthermore, in this study, there was no significant interaction between inbreeding and physical enrichment on juveniles' general activity, which questions the effects of different types of enrichment on behaviour. Interactions involving inbreeding (either with enrichment, whether social or physical or both, or activity), showed different effects in juvenile versus adult mice, as well as between generation. These results highlight that although there is consistently an effect of inbreeding, more work is needed to understand the ontogenetic and inter-generational effects reported here.

2.5.2 Effects of enrichment and other factors on activity and stereotypy

Enrichment per se had several effects on activity or stereotypy in mice. For instance, social enrichment significantly affected general activity in the home cage of both G_1 and G_2 mature individuals (as seen above, social enrichment also affected activity in juveniles, however through an interaction with inbreeding), but not in a straightforward way: the direction of the effect depended on the generation (Table 2.3). In G_1 mice, activity increased with an increasing number of individuals, while it decreased in G_2 mice. Currently, we lack sufficient data and knowledge to disentangle possible causes of these phenotypic differences and interpret these seemingly contradictory results, as enrichment generally stimulates play behaviour and general activity in the home cage (Marashi et al. 2004), whereas social isolation may cause 'depressive-like' behaviours. Research has also shown that social and/or physical enrichment increase activity/exploration by decreasing fearfulness in fear-inducing situations such as open-field tests or novel environments (Gardner et al. 1975; Kohl et al. 2002; Jones 2004), but research on general activity in the home cage is lacking. The fact remains nonetheless that despite evidence for individual consistent differences in general activity levels in this study, some important extrinsic sources of variation such as number of cage mates can affect the activity of an individual. If environment influences activity levels, but activity has a reverse effect on stereotypy, then it is possible to observe no relationship between environment and stereotypy. We tried to tease apart these possible confounding effects by first conducting analyses of enrichment effects on activity levels, and then developing a different model integrating enrichment and activity effects on stereotypy.

Indeed, a significant social enrichment by activity interaction was observed in juveniles from both generations such that stereotypy increased with an increasing activity and a decreasing number of individuals (Fig. 2.4). In other words, social enrichment was most beneficial to active animals because it provided them with alternative activities to stereotypy. Interactions with conspecifics may serve as a physical outlet and inhibit repetitive behaviours in active individuals. Also, deer mice are gregarious animals, and lack of social interactions among active animals following weaning — the time when social play usually develops — may increase stress and induce stereotypy. Studies in rats show, for instance, that social deprivation can lead to stereotypic behaviours (Sahakian et al. 1975). Rats kept in isolation following weaning suffered from altered brain development (notably in the prefrontal cortex), which affected anxiety-related behaviours (Robbins et al., 1996; see review by Hall 1998). They also showed a tendency to perform non-appropriate repetitive behaviours such as stereotypy (Balcombe 2006). However, if in G_1 and G_2 juveniles, stereotypy was less pronounced in socially enriched active mice than in socially deprived active mice, contrary to our expectations adult active mice did not show a different incidence of stereotypic behaviours in relation with social enrichment, which suggests that social enrichment can affect the onset and early maintenance of stereotypic behaviours in individuals, but not the later presence of stereotypy.

It must be said here that general activity increased with age in both G_1 and G_2 juveniles irrespective of environment. Interestingly, contrary to social enrichment, physical enrichment did not affect activity of juveniles or of mature individuals, which suggests that activity is independent of the physical environment. One would expect that, because the enrichment chosen in this study favoured elements of novelty and aimed at stimulating interest towards the cage environment and providing an outlet for locomotor activity (tunnels that changed every week, unlimited access to running wheels, etc.), physical enrichment would promote activity; our results indicate that this is not the case. If the weekly unpredictable change of the internal space of the cage did impact activity levels, it was too short-lived to be observed.

These findings are important in that they show that, while more active individuals are more strongly stereotypic when reared in a standard environment, this is not the result of an underlying (and confounding) effect of environment on the level of activity. Indeed, physical

enrichment affected stereotypy through an interaction with activity (Fig. 2.5): in G_1 and G_2 pups and both G_1 and G_2 mature individuals, stereotypy increased much more steeply with activity in standard cages than in enriched cages. These results suggest that neither environment nor activity levels have a straightforward effect in eliciting or exacerbating stereotypy in captive animals inasmuch as less active animals from both environments do not tend to become stereotypic (which is not surprising since to perform stereotypy, animals must be active), while active animals benefit from a physical enrichment because it allows them to spend their active time on other forms of activity than stereotypy. This study provides evidence that enrichment has a differential effect on mice's stereotypy and thus may reduce or prevent development of stereotypic behaviours in active individuals. It raises an important question relative to the effectiveness of commercially available enrichments in helping prevent stereotypic behaviours. It has been suggested that commercial enrichment items have not been properly assessed or demonstrated to be beneficial to mice. Garner (2005) points out that "for an enrichment to be enriching, it must be 'biologically relevant' (i.e. it must address a behavioral need and facilitate the performance of highly motivated behaviors)", and that, although toys may be relevant for primates, they may not be so for mice (Olsson & Dahlborn 2002). Also, shelter provided by tunnels, igloos and complex cages may benefit some mice, but induce territoriality and hence be deleterious to others, especially males housed in groups (Olsson & Dahlborn 2002). We show here that commercial enrichment particularly benefits very active mice.

2.5.3 Family- and individual-level variation in activity and stereotypy

Family was a significant factor affecting activity and stereotypy levels in all analyses except in G_1 juveniles' activity. This indicates that between-family variance was greater than within-family variance, i.e. siblings were more likely to be similar for the measured variables (activity and stereotypy levels) than non-relatives. Strong family effects may reflect genetic (besides inbreeding), maternal, or common environment effects (Kruuk 2004), which cannot be disentangled in the present model; because fathers were separated before birth or at parturition, their phenotypes could not have directly affected the phenotypes of their

offspring. Significant maternal effects indicate that the care and environment a mother provides to her offspring influence their phenotype, hence increasing the resemblance among offspring of the same family (Kruuk 2004). Deficits in maternal behaviour may enhance development of stereotypic behaviours in offspring (Berkson 1967, Harlow et al. 1965, cited in Powell et al. 2000). Studies on rats suggest that variation in maternal care can result in a non-genomic transmission ("epigenetic programming") of responses to stress (Weaver et al. 2004), hence affecting stress levels of progeny and predisposing individuals of a same family to perform stereotypies (in Würbel 2001). Furthermore, stereotypy may be learned from a stereotypical mother: e.g. pigeons (*Columba livia*) seemed to copy stereotypic behaviours from nearby conspecifics and engaged in stereotypies even in the absence of their 'tutors' (Palya & Zacny 1980, cited in Mason 1991). Strong family effects may also reflect a common environment: individuals from a same family shared an environment and this may have increased their chances of having similar phenotypes (Kruuk 2004). In our study, there was also a significant effect of individual on activity and stereotypy, which indicates between-siblings differences. In our model, methods used to estimate maternal effects are only able to detect variation in a given trait between offspring of different mothers (Kruuk 2004). However, individual effects can include maternal effects specific to the individual but not affecting its siblings. Mothers may also affect each of their pups differently, depending on their characteristics (Lesselis 2002; Badyaev et al. 2003); the reverse is also true. Individual effects also suggest within-family genetic variation. Each individual has a unique genetic background, hence idiosyncrasies of offspring in the reaction to eliciting stimuli (thresholds to stress, sensitivity, etc.) (Mason 1991; Schoenecker & Heller 2000).

Individual adult mice were consistent in their activity and in their stereotypic behaviour over time, with r ranging between 0.63 and 0.77. In contrast, juveniles were not always consistent in their activity levels ($r = 0.04$ and 0.23 in G_1 and G_2 respectively) while they were consistent in their stereotypic behaviours ($r = 0.64$ and 0.67 in G_1 and G_2 respectively) once correcting for variations in activity levels between observations. This result is surprising given that observations were made during the ontogeny of stereotypy, i.e. before individuals had acquired a definite stereotypy profile. Individual effects did become stronger with age,

indicating that individuals became more consistent in their behaviour, and less affected by 'Family' (see Tables 2.3 and 2.4). Individual behavioural differences could not be explained by number of individuals per cage, physical enrichment and inbreeding status, as those effects are accounted for in the models as fixed effects. A high repeatability indicates that the variation observed at the phenotypic level was not caused by temporary conditions, confirmed by the fact that juvenile and adult deer mice's stereotypy was strongly correlated, and the same holds true for activity in young and adult G_2 individuals, but not for G_1. These results indicate that under constant conditions, individuals show some consistency in their stereotypy levels throughout their development. However, individuals' stereotypy levels are not fixed, with some individuals showing high stereotypy as juveniles and low stereotypy as adults, and vice versa. Furthermore, a repeatability exceeding 0 indicates that additive genetic variance may be at work and that the behaviours studied can be heritable (Kruuk 2004). However, family effects make up a limited percentage of variance related to random effects.

2.6 Conclusions

A fundamental assumption underlying this study is that locomotor stereotypy indicates that an animal shows limited behavioural adjustments to a captive environment and hence can be considered as an indicator of stress (Moberg 2000). One of the aims of enrichment is to allow animals to perform species-typical behaviors that give them control over the environment and promote homeostasis (Olsson & Dahlborn 2002). In a laboratory experimental setting, an environment is considered enriched (compared to standard housing conditions) when animals are housed in bigger and more complex cages that provide the opportunity for voluntary physical activity (for example, on running wheels), and/or social interactions (for social animals). In this study, as expected, the standard cages without a rubber pipe were detrimental to G_1 inbred juveniles, who exhibited more stress-related behaviours (as indicated by stereotypy) than enriched outbreds. However, once a small pipe was added in the standard environment, inbreds and outbreds no longer differed with respect to stereotypy. This may have resulted because the addition of the pipe made the standard environment more benign, particularly given the fact that the outbred mice had low (non-

zero) levels of inbreeding. Also, there may be different behavioural responses to stress, and the type of response may depend on the duration and intensity of the stress (Hoffmann & Parsons 1991). Poor social and physical conditions can affect phenotypes, but these stresses do not necessarily lead to stereotypy and could vary with genotypic background. It is likely that behavioural traits are influenced by inbreeding in much the same way as other traits, although research in this field is scant and more studies need to address their relationship. The fact remains nonetheless that even though the physically enriched cages used in our study were far from insuring a naturalistic environment to the mice, they benefitted active animals.

Differences in general activity and stereotypic behaviours of mice were observed throughout the lifetime of individuals in relation to environmental (physical and social enrichment) or intrinsic (age, inbreeding) conditions. However, the same conditions had different effects with regards to age. There are several possible reasons for this. It could be hypothesized that some categories of mice were affected earlier by some conditions, while other categories of individuals underwent later changes in their phenotypes in the same direction, which levelled out variation between adult individuals. More importantly, it may indicate that adult deer mice have become impervious to conditions that determined their behaviour earlier in life. Whatever the reasons, it seems that juveniles are more sensitive to prevailing conditions, as they are sensitive to a wider range of effects than older mice; thus, examining effects only at a single life-history stage (i. e., during the juvenile period) may result in underestimation or overestimation of the extent of environmental effects and/or inbreeding depression. Our longitudinal behavioural data help us address genetic and enrichment contributions to change or continuity. Moreover, individuals from the same family resemble each other more than non-related individuals regarding stereotypic locomotor behaviours and activity, however substantial variation still exists between individuals within families.

CONCLUSION

Nous nous proposions d'étudier les effets de la consanguinité et de l'enrichissement sur la stéréotypie et sur l'activité de souris sylvestres maintenues en captivité. Nous avons montré que les souris G_1 juvéniles qui étaient consanguines et maintenues en milieu standard avaient effectivement une plus grande propension à devenir stéréotypiques que les souris consanguines maintenues dans un milieu enrichi et que les souris non consanguines des milieux standard et enrichi. Cependant, cet effet n'était plus observé chez les mêmes individus devenus adultes et bénéficiant d'un élément d'enrichissement en plus (un simple tube de caoutchouc pouvant servir de refuge). Il n'a pas non plus été relevé chez les souris de la génération suivante. Il est possible que l'ajout d'un simple tuyau pouvant servir de refuge ait répondu à un besoin biologique des souris sylvestres si important qu'il a abaissé de façon marquée le niveau de stress des individus consanguins. Il est aussi possible que la consanguinité n'influe pas nécessairement et de façon permanente sur les comportements à l'étude. Des recherches montrent que ses effets sur la fitness peuvent varier selon le stade de développement des animaux (Kalinowski *et al.* 1999 ; Wilcken 2001 ; Vermeulen et Bijlsma 2004). Les souris consanguines juvéniles sont peut-être plus sensibles aux conditions ambiantes que les souris adultes. Les études longitudinales portant sur l'effet d'un stress environnemental sur le comportement sont très rares et, à ce stade-ci de nos connaissances, il est difficile de tirer des conclusions probantes sur l'origine de cette variation temporelle. Même chose en ce qui concerne les différences intergénérationnelles. Toutes les souris consanguines de notre étude étaient issues d'un croisement frère-sœur, donc avaient un coefficient de consanguinité similaire. Il est difficile de croire que, aléatoirement, nous ayons produit une deuxième génération de souris plus résistante au stress (tel qu'indiqué par les comportements stéréotypiques). Il est possible que le degré de consanguinité des souris n'ait pas été assez élevé pour que les individus consanguins manifestent une plus grande susceptibilité au stress, mais il est plus probable que la « faible » consanguinité inévitable des souris dites non consanguines reproduites en laboratoire ait nivelé la susceptibilité au stress des individus à l'étude. Cependant, nous ne pouvons que nous livrer à des conjectures à ce sujet et suggérer de répliquer cette étude en produisant des souris consanguines dont le coefficient de consanguinité varierait de façon plus continue entre 0,1 et 0,25. Nous avons

bien essayé de croiser les souris en tenant compte de cela, mais la reproduction des individus consanguins ou issus de consanguins en milieu standard était si faible au terme de plusieurs mois que cela n'a guère été possible. Il serait bon aussi de créer des milieux dont l'enrichissement serait plus continu ou, du moins, de reproduire une recherche intégrant deux types de milieu standard : l'un vraiment pauvre, comme celui dans lequel ont grandi les souris juvéniles de la G_1 (malheureusement très commun dans les laboratoires), et un autre dit « standard » intégrant en plus un simple élément de refuge, comme celui dont ont bénéficié les souris matures de la G_1 et tous les individus de la G_2.

L'interaction entre la consanguinité et l'enrichissement social sur l'activité générale dans la cage s'est révélée significative dans le cas des souris juvéniles des deux générations : l'activité des individus non consanguins était bien supérieure à celle des consanguins lorsque le nombre d'individus par cage était élevé, mais l'inverse était observé à un faible enrichissement social. Cependant, il n'est pas facile d'interpréter lesdits résultats, car un examen plus attentif des courbes montre que la direction de celles-ci n'est pas cohérente entre les générations. Il reste, toutefois, que dans les deux cas la consanguinité interagit avec l'enrichissement social de sorte à affecter l'activité des juvéniles des deux générations. Par ailleurs, cette interaction n'est plus observée chez les adultes des deux générations, dont le niveau d'activité générale dans la cage n'était plus influencé significativement que par l'enrichissement social. Précisons ici que l'effet de la consanguinité sur le niveau général d'activité a été très peu étudié. Une recherche sur le sujet menée par Margulis (1997) auprès d'une autre espèce de souris indique que les individus consanguins sont moins actifs que les non-consanguins. Toutefois, à ce stade-ci de nos connaissances, il n'est pas possible d'expliquer ces différences phénotypiques et d'interpréter ces résultats qui ne concordent pas entièrement avec les nôtres, dans la mesure où l'enrichissement social n'a pas été pris en compte dans l'étude de Margulis. Enfin, nous nous serions attendus à ce que les souris actives de notre étude aient eu une plus grande propension à devenir stéréotypiques que les souris moins actives mais, ne connaissant pas assez les effets de la consanguinité sur l'activité générale, il nous était difficile de prédire l'effet d'une interaction entre la consanguinité et l'activité sur la stéréotypie. Les résultats relatifs aux adultes de la G_2 indiquent que la consanguinité peut interagir avec le niveau d'activité et affecter les comportements

stéréotypiques : ainsi, les souris consanguines très actives de la G_2 adulte ont une plus faible propension à faire de la stéréotypie que les souris très actives non consanguines. À ce point-ci des connaissances sur le sujet, il est difficile d'interpréter ces résultats sans recourir à des conjectures. Dans cette étude, l'expression de l'activité et son intensité n'ont pas été incluses dans les analyses. Des souris peuvent avoir un niveau d'activité comparable dans la mesure où elles ne sont pas immobiles pendant la même proportion de temps, mais l'énergie dépensée en courant ou en faisant de la stéréotypie n'est certes pas la même que celle qui est perdue en reniflant les coins d'une cage, en buvant ou en mangeant. Il serait bon, donc, de tenir compte de l'intensité de l'activité des individus.

L'enrichissement physique ou social a affecté l'activité ou la stéréotypie des souris de différentes façons. Ainsi, l'activité des souris matures de la G_1 croissait quand le nombre d'individus augmentait (indépendamment du milieu physique), alors que celle de la G_2 adulte diminuait quand le nombre d'individus augmentait (chez les juvéniles, comme nous l'avons vu précédemment, l'enrichissement affectait l'activité mais en interaction avec la consanguinité). Ainsi, le niveau d'activité des souris sylvestres ne semble pas intrinsèque dans la mesure où il est modulé par l'enrichissement social. Des études indiquent que l'enrichissement physique et/ou social stimulent le jeu et l'activité générale dans l'environnement habituel (soit la cage) (Marashi *et al.* 2004), ainsi que l'activité et l'exploration dans un nouvel environnement (Jones 2004; Kohl *et al.* 2002), alors que les effets combinés d'un appauvrissement physique et d'un isolement social sur l'activité générale sont moins étudiés, mais indiquent une décroissance de l'activité. Cela va en partie dans le sens des résultats que nous avons obtenus. On s'attendrait à ce qu'un enrichissement social effectué dans un milieu relativement grand et complexe stimule l'activité, mais qu'un grand nombre d'individus enfermés dans un milieu restreint inhibe l'activité d'au moins quelques individus. Il est donc difficile de comprendre pourquoi l'enrichissement social a affecté l'activité des souris des deux générations de façon contradictoire et indépendante de l'enrichissement physique. Par ailleurs, en ce qui a trait à la stéréotypie, notre étude indique que l'enrichissement social touchait différemment les individus, en fonction de leur niveau d'activité : ce sont les souris les plus actives qui bénéficiaient le plus d'un enrichissement social. Les souris peu actives, encagées seules ou non, avaient une plus faible propension à

développer des comportements stéréotypiques, ce qui est attendu, étant donné que, pour faire de la stéréotypie, il faut être actif.

Par ailleurs, contrairement à l'enrichissement social, l'enrichissement physique n'a pas affecté de façon significative le niveau d'activité des souris juvéniles ou matures à l'étude, ce qui indique que le niveau d'activité générale dans la cage ne dépend pas du milieu physique environnant. Cela semble étonnant si l'on tient compte du fait que le milieu enrichi était renouvelé hebdomadairement (changement du nombre et de la structure des tunnels, de leur disposition spatiale — horizontale ou verticale —, du nombre d'igloos, etc.) de sorte à stimuler l'intérêt des souris y vivant. Par contre, les souris les plus actives du milieu standard avaient une plus grande propension à développer des comportements stéréotypiques que les souris les plus actives du milieu enrichi. Les résultats obtenus dans cette étude soulèvent la question de l'enrichissement en milieu captif : ainsi que le relève Garner (2005), pour qu'un enrichissement soit enrichissant, il doit répondre à des besoins biologiques et permettre d'effectuer des comportements fortement motivés. Toutes les souris de notre étude (hormis les G_1 juvéniles) bénéficiaient d'une forme d'enrichissement physique appropriée aux souris (du coton pour le nid et au moins un tuyau ou un tunnel pour s'abriter). Ce qui ressort ici, c'est que l'enrichissement ne bénéficie pas de la même façon à tous les individus : il est plus efficace (il diminue la propension à être stéréotypique) dans le cas des individus actifs.

Notons à cette étape-ci que les effets des conditions environnementales (enrichissement social, enrichissement physique) ou internes (consanguinité) sur l'activité et sur la stéréotypie varient selon l'âge. On pourrait penser que certaines conditions de captivité (ex.: la consanguinité en milieu pauvre) font qu'un comportement se développe plus précocement mais que, au fil du temps, l'écart phénotypique interindividuel décroît. Une autre hypothèse serait que les souris adultes deviennent moins perméables à des conditions qui ont modelé leurs comportements à une étape antérieure de leur vie. Nous nous proposons de le vérifier ultérieurement en nous servant de notre base de données pour procéder à des analyses complémentaires.

Une quantité innombrable d'animaux est maintenue en captivité, que ce soit en laboratoire, dans des fermes d'élevage ou autres. Beaucoup sont consanguins, et beaucoup

vivent dans des conditions de dénuement social ou physique. Que ce soit pour accroître leur bien-être, pour standardiser les études fondées sur un modèle animal ou tout simplement pour améliorer nos connaissances fondamentales, il serait utile d'approfondir cette recherche. Il nous semble particulièrement important de réaliser une étude à plus grande échelle, sur plusieurs générations et de façon plus systématique qui permettrait de départager les effets de l'enrichissement social et de l'enrichissement physique sur l'activité, et sur l'apparition et le maintien de comportements stéréotypiques. Une fois cela fait, on intégrerait les effets environnementaux et le tempérament dans l'étude du développement et du maintien des comportements stéréotypiques. Dans cette étude, au moment du sevrage, les individus d'une même famille étaient séparés en fonction du sexe. Les individus d'une famille nombreuse avaient donc moins de chances de se retrouver seuls, à moins d'une sex-ratio biaisée. En outre, l'échantillon de souris seules était plus grand en milieu riche qu'en milieu pauvre, les souris du milieu pauvre s'étant reproduites plus tard ; cela était particulièrement accentué dans le cas des souris consanguines. Il est fort possible que, en raison de cela, l'analyse de l'enrichissement social ait manqué de puissance statistique. Pour ce qui est de l'enrichissement physique, il pourrait être effectué de sorte à créer un environnement plus naturel.

Enfin, notre étude montre que les individus d'une même famille se ressemblent plus qu'ils ne ressemblent à ceux des autres familles sur le plan de l'activité et des comportements stéréotypiques. Des effets de familles significatifs reflètent des effets maternels et/ou génétiques (Kruuk 2004). Il a déjà été montré que l'activité et la stéréotypie pouvaient être héritables. Cela reste à corroborer, par exemple à l'aide d'un modèle plus complexe, comme un modèle animal, qui nous donnerait la possibilité d'utiliser une seule base de données intégrant les deux générations. Du même coup, nous accroîtrions notre échantillon et pourrions bénéficier d'une plus grande puissance statistique. Enfin, si l'activité et les comportements stéréotypiques sont influencés par l'appartenance à la famille, ils sont aussi très influencés par des réactions idiosyncratiques de l'individu. Avec l'âge, les effets de famille et la variance résiduelle décroissent, à mesure que les individus deviennent plus constants, ce qui soulève des questions au sujet de la fixation de la stéréotypie à un jeune âge lorsque les conditions demeurent inchangées.

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